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GW25-e3573

Rb1 Protects Endothelial Cells from Hydrogen Peroxide-Induced Cell Senescence: Involvement of Caveolin-1

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Objectives: Endothelial senescence has been proposed to be involved in endothelial dysfunction and atherogenesis. This study investigates the effects of ginsenoside Rb1, a major constituent of ginseng, on H_2O_2-induced endothelial senescence. And here we have investigated the expression and production of caveolin-1, a protein that has been shown previously to be upregulated in stress-induced premature senescence.
Methods: Primary human umbilical vein endothelial cells (HUVECs) senescence was induced by H_2O_2 as judged by senescence-associated β-galactosidase assay (SA-β-gal). Caveolin-1 mRNA expression was analyzed by real time PCR. Caveolin-1 protein expression was determined by Western blot and laser scanning confocal microscopelaser scanning confocal microscopy.
Results: Treatment of HUVECs with 60μM H_2O_2 induced premature senescence. Pretreatment of HUVEC with Rb1 was found to reverse endothelial senescence, as witnessed by a significant decrease of senescent cell numbers (approximately 2-fold reduction). Rb1 could markedly decrease Caveolin-1 mRNA expression compared with cells treated with H_2O_2 alone. Meanwhile, Caveolin-1 protein expression decreased in the 20μM Rb1-pretreated cells compared to that in cells treated with H_2O_2 alone. By laser scanning confocal microscopy, we also found that Rb1 can effectively decrease Caveolin-1 protein expression.
Conclusions: Our report demonstrates that Rb1 can exert reversal effects on H_2O_2-induced cellular senescence through modulating Caveolin-1 expression.

GW25-e4120

IGF-1 Inhibits Apoptosis of Vascular Smooth Muscle Cells Through PI3/Akt Pathway

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Objectives: Apoptosis of vascular smooth muscle cells (VSMCs) has recently been identified as an important process in a variety of human vascular diseases, including atherosclerosis. Apop-1, a novel gene identified in cultured atherosclerotic smooth muscle cells of ApoE-deficient mouse and is known to induce apoptosis in several cells, including VSMC. Insulin-like growth factor (IGF-1) and platelet-derived growth factor (PDGF) are well characterized survival factors for VSMC. However, the interaction between the pro-apoptotic protein Apop-1 and survival factors IGF-1 and PDGF on mediation of apoptosis in VSMC are poorly understood.
Methods: Immunocytochemistry; For immunocytochemistry analysis, cells were seeded onto glass coverslips. After transiently transfected with the vector encoding Apop-1-GFP and further incubated, the cells were fixed with 4% paraformaldehyde in PBS for 20 min. Washing the cells were incubated for 6h, 24h at 4 °C with anti-cytochrome c mAb, washed twice in PBS and developed with rhodamine-conjugated goat anti-mouse antibodies. DNA analysis of apoptosis by flow cytometry. Apoptosis was monitored by measuring the population distribution of DNA content. The DNA content of the cells was analysed by flow cytometry on FL2 channel.

Western immunoblot analysis; Cells were washed with phosphate-buffered saline (PBS) twice and harvested by scraping. From 20ug of protein extract was fractionated on SDS-PAGE (polyacrylamide electrophoresis gel) and transfected to a polyvinylidine difluoride membrane (Millipore, Burlington, MA). After three washes with T-PBS, the membrane was incubated with secondary antibody (anti-rabbit IgG-HRP conjugated, anti-mouse IgG-HRP conjugated and anti-goat IgG-HRP conjugated.) for 1 h, and then washed with 0.05% Tween 20 in PBS. The immune complexes were detected by chemiluminescence methods (ECL, Amersham, Piscataway, NJ).

Caspase-9 assays; Caspase-9 activity was measured using the caspase-9/Mch6 fluorometric protease assay kit according to the directions of the manufacturers (BioVision). In brief, cell lysates prepared from 1×106 cells were incubated with 10mM DTT and a caspase-9-specific substrate LEHD-AFC in reaction buffer for 1.5 h at 37 °C.
Results: In this report, we show that the signaling cascade involved in IGF-1 protectes VSMC against Apop-1-induced apoptosis, while PDGF has no effect. In addition, pretreatment of Apop-1 transfected VSMCs with phosphatidylinositol-3-kinase inhibitor wortmannin, or infection with an adenoviral construct expressing the dominant negative Akt gene (Adeno-dnAkt) blocked the cytoprotective effect of IGF-1, whereas the MEK inhibitor PD98059 had no effect. Conversely, infection with an adenoviral construct expressing the constitutively active Akt (Adeno-MyrAkt) gene, protected VSMC from apoptosis induced by Apop-1 even in the absence of IGF-1, suggesting that IGF-1 prevents VSMC apoptosis induced by Apop-1 through activation of the PI3K/Akt pathway. Furthermore, IGF-1 elevated phospho-Akt expression in Apop-1 transfected VSMCs and Apop-1 decreased phospho-Akt expression. Importantly, IGF-1 inhibited cytochrome c release from mitochondria and blocked activation of intrinsic initiator caspase-9 in Apop-1 transfected VSMCs.
Conclusions: These findings suggest that inhibition of Apop-1-induced apoptosis by IGF-1 is via promotion of Akt activation through PI3K/Akt signaling pathway which may contribute to stabilize atherosclerotic plaque in patients with atherosclerosis.

GW25-e4137

The Effects of H_2O_2 on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel Current and its Mechanisms in Neonatal Rat Cardiomyocytes

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Objectives: To identify the effects of exogenous hydrogen peroxide on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel (HCN) current and its mechanisms in Neonatal Rat Ventricle Cardiomyocytes (NRVM).
Methods: NRVM from 1- to 3-day-old Wister rats were prepared by collagenase digestion, and incubated in 37°C, 95%CO_2 for patch-clamp recording. HCN channel protein expression was detected by western-blotting analysis.
Results: Our data shown that exposure (∼20 min) of NRVM to H_2O_2 (100 μmol/L) markedly increased I_f density (4.7±0.6 pA/pF vs. 11.7±1.1 pA/pF) along increased conductance (G_{max}: 48.7±5.6 pS/pF vs. 192.6±64.1 pS/pF), a shift in activation voltage ($V_{1/2}$) to positive potentials (-81.2±1.6 mV vs. -64.7±2.0 mV) and increase rate of activation (tact) (523.4±24.7 ms vs. 337.5±24.9 ms). Moreover, stimulation by H_2O_2 was largely inhibited by the non-specific tyrosine kinase blocker genistein (1μmol/L) or the c-Src-specific inhibitor PP2 (10 μmol/L). Augmented tyrosine phosphorylation of HCN2 channels with H_2O_2 treatment by determined by H_2O_2 Western blot using the phosphotyrosine specific antibody 4G10. Furthermore, the augmented I_f current was inhibited by pre-treatment with Trx receptor inhibitor (Auranofin 10nmol/L; 13-cis-retinoic acid 1 μmol/L). On the other hand, I_f current of NRVMs was also increased by treated with non-specific PTP inhibitors, phenylarsine oxide (PAO 1 μmol/L) or Na-orthovanadate (Na_3VO_4 10 μmol/L).
Conclusions: hese data suggest that the c-Src family of tyrosine kinase mediate the augmentation of I_f density by oxidant agent H_2O_2 via a redox mechanism involving the Trx system.

GW25-e4157

Embodiment of Therapeutic Principle of TCM in "Arrhythmia Emergency Dealing with General Principles"

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Objectives: As an important part of the basic theory of TCM, the therapeutic principle of TCM guide the clinical treatment of TCM in different angles and different levels. To guide the clinical treatment of arrhythmia of integrated traditional Chinese and western medicine, grasp of the clinical application and significance better.
Methods: This article analysis the arrhythmia emergency dealing with general principles by the therapeutic principle of TCM.
Results: In the "Arrhythmia emergency dealing with general principles", the first, identify and correct the hemodynamic disorder reflect the "specimen emergency" in the therapeutic principle of TCM; The second, correct basic diseases and incentive reflect the "treatment aiding at the root cause of disease" in the therapeutic principle of TCM; he third, measure the benefit and risk reflect the "as the goal of smooth, neutral thinking" in the therapeutic principle of TCM; The fourth, balance the treatment and prevention reflect the "preventive treatment of diseases" in the therapeutic principle of TCM; The fifth, in the treatment of arrhythmia itself, slow ventricular rate to stable condition reflect the "homotherapy for heteropathy" in the therapeutic principle of TCM; The sixth, the drug application principle in acute phase of antiarrhythmic reflect the "treating diseases in accordance with the patient's constitution and treatment by differentiation of syndromes" in the therapeutic principle of TCM.
Conclusions: This article analysis the arrhythmia emergency dealing with general principles by the therapeutic principle of TCM, which can grasp of the clinical application and significance better, and has reference value for the clinical treatment of arrhythmia of integrated traditional Chinese and western medicine.

GW25-e4203

The effect of angiotensin II receptor type 1 autoantibodies on fetal rats' cardiac hypertrophy and underlying mechanisms

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Objectives: The local renin angiotensin system (RAS) is an independent risk factor which can promote the fetal myocardial hypertrophy. AT1-AA, the autoantibody of the angiotensin II (Ang II) type 1 receptor (AT1R), was existed in preclamptic women and might be a new pathological factor that induced fetal myocardial hypertrophy. However, the specific mechanism is still unclear. This study aims to investigate